

Att. Docket No. REG 753B
USSN 10/061,043
Response to Notice to File Missing Parts
of Nonprovisional Application

REMARKS

This amendment is made merely to insert the sequence identifiers into the specification and to correct some minor typographical errors.

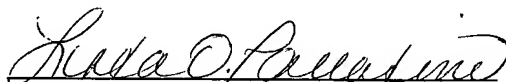
Applicants direct the subject Sequence Listings submitted concurrently be added to the specification.

I hereby state that the content of the paper and computer readable copies of the Sequence Listing, submitted concurrently in accordance with 37 C.F.R §1.821(c) and (e) respectively, are the same. I hereby state that the content of the paper and computer readable copies of the Sequence Listing submitted concurrently and referred to herein in accordance with 37 C.F.R. § 1.821(g), contain no new matter.

FEES:

No fee other than \$130.00 fee is deemed necessary in connection with the filing of this Response. However, if any additional fee is required, authorization is hereby given to charge the amount of any such additional fee to Deposit Account No. 18-0650.

Respectfully submitted,



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MARKED-UP VERSION SHOWING CHANGES

Paragraph starting on page 3, line 6:

Protein degradation is a common mechanism used by cells to control protein abundance. However, rather than simply degrading all proteins, ubiquitination seems to be very specific in terms of protein target selection. The formation of such ubiquitin-protein conjugates involves a protein complex consisting of three components: a ubiquitin activating enzyme (E1), a ubiquitin conjugating enzyme (E2), and a substrate specificity determining component (E3) (Skowyra, et al, 1997, Cell 91:209-219). There are several distinct molecular strategies that regulate which protein targets become ubiquitinated. A recently discovered mechanism is referred to as the SCF E3 ubiquitin ligase complex (see Figure 1 for a schematic representation of the complex). The SCF protein complex comprises several distinct protein subunits, including a protein which has a domain referred to as an "F-box." In the presence of a phosphorylated substrate, the SCF complex binds to the substrate, and ubiquitinates it, using an E2 ubiquitin transferase which is also part of the SCF complex (Patton, et al, 1998, Genes & Development 12:692-705). The result is the specific proteolytic degradation of the substrate. F-box proteins comprise a large family that can be divided into three subfamilies: 1) Fbws, which are characterized by multiple Trp-Asp repeats (WD-40 repeats); 2) Fbls, which are characterized by leucine-rich repeat; and 3) Fbxs, which lack known protein interaction domains (see Winston, et al, 1999, Current Biology 9:1180-1182 for a discussion of the currently known mammalian F-box protein family members). F-box proteins usually contain an additional substrate-binding domain that interacts with specific protein substrates and a 42-48 amino acid motif termed the F-box (Winston, 1999). See Figure 2 (SEQ ID NOS: 6-15) for a comparison of hMAFBX with other F-box-containing proteins.

Paragraph starting on page 5, line 23:

The invention additionally describes a novel protein-protein interaction domain of MA-61. This domain was determined by comparing the MAFBX protein to a previously discovered F-box-containing protein, Fbx25. These two proteins contain an area of homology distinct from the F-box domain. Applicant calls this domain the Fbx25 homology domain. See Figures 5A-5B (SEQ ID NOS: 16-19) for the comparison of MAFBX with Fbx25.

Paragraph starting on page 9, line 28, through page 10, line 3:

The invention further provides for a method for screening for agents useful in the treatment of a disease or disorder associated with muscle atrophy comprising contacting a cell expressing MURF1, MURF3 or MAFBX having the amino acid sequence of Figure 7 (SEQ ID NO: 21), Figure 9 (SEQ ID NO: 23), Figure 11 (SEQ ID NO: 25), Figure 13 (SEQ ID NO: 27), Figure 17 (SEQ ID NO: 33), Figure 19 (SEQ ID NO: 35), and Figure 22 (SEQ ID NO: 39), respectively, or a fragment thereof, and its substrate, with a compound and detecting a change in the activity of either MURF1, MURF3, or MAFBX gene products. Such change in activity may be manifest by a change in the interaction of MURF1, MURF3, or Mafbx gene products with one or more proteins, such as one of their substrates or a component of the ubiquitin pathway, or by a change in the ubiquitination or degradation of the substrate.

Paragraph starting on page 12, line 28:

Figure 2 (SEQ ID NOS: 6-15): Sequence comparison demonstrating F-box domain of MA-61 (SEQ ID NOS: 6-15).

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Paragraph starting on page 13, line 1:

Figures 5A-5B (SEQ ID NOS: 16-19): Sequence comparison between MAFBX and Fbx25 showing broad homology (SEQ ID NOS:16-19).

Paragraph starting on page 13, line 4:

Figure 6 (SEQ ID NO: 20): Nucleotide sequence of rat MURF1 (SEQ ID NO: 20).

Paragraph starting on page 13, line 6:

Figure 7 (SEQ ID NO: 21): Deduced amino acid sequence of rat MURF1 (SEQ ID NO: 21).

Paragraph starting on page 13, line 8:

Figures 8A-8C (SEQ ID NO: 22): Nucleotide sequence of human MURF1 (SEQ ID NO: 22).

Paragraph starting on page 13, line 10:

Figure 9 (SEQ ID NO: 23): Deduced amino acid sequence of human MURF1 (SEQ ID NO: 23).

Paragraph starting on page 13, line 12:

Figure 10 (SEQ ID NO: 24): Nucleotide sequence of rat MAFBX (SEQ ID NO: 24).

Paragraph starting on page 13, line 14:

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Figure 11 (SEQ ID NO: 25): Deduced amino acid sequence of rat MAFBX (SEQ ID NO: 25).

Paragraph starting on page 13, line 16:

Figure 12 (SEQ ID NO: 26): Nucleotide sequence of human MAFBX clone K8 (SEQ ID NO: 26).

Paragraph starting on page 13, line 18:

Figure 13 (SEQ ID NO: 27): Deduced amino acid sequence of human Mafbx clone K8 (SEQ ID NO: 27).

Paragraph starting on page 13, line 20:

Figure 14 (SEQ ID NOS: 28-31): Sequence comparison demonstrating ring domain of MURF1 (SEQ ID NOS: 28-31).

Paragraph starting on page 13, line 25:

Figure 16 (SEQ ID NO: 32): Nucleotide sequence of rat MURF1 VRV splice form (SEQ ID NO: 32).

Paragraph starting on page 13, line 27:

Figure 17 (SEQ ID NO: 33): Deduced amino acid sequence of rat MURF1 VRV splice form (SEQ ID NO: 33).

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Paragraph starting on page 13, line 29:

Figure 18 (SEQ ID NO: 34): Nucleotide sequence of human Mafbx clone D18 (SEQ ID NO: 34).

Paragraph starting on page 13, line 31:

Figure 19 (SEQ ID NO: 35): Deduced amino acid sequence of human Mafbx clone D18 (SEQ ID NO: 35).

Paragraph starting on page 13, line 33:

Figure 20 (SEQ ID NOS: 36 and 37): Sequence alignment of rMURF1 with hMURF3 (SEQ ID NOS: 36 and 37).

Paragraph starting on page 14, line 1:

Figure 21 (SEQ ID NO: 38): Nucleotide sequence of human MURF3 clone C8 (SEQ ID NO: 38).

Paragraph starting on page 14, line 3:

Figure 22 (SEQ ID NO: 39): Deduced amino acid sequence of human MURF3 clone C8 (SEQ ID NO: 39).

Paragraph starting on page 14, line 13:

Figure 26 (SEQ ID NOS: 40-42) Sequence alignment of rat (SEQ ID NO: 40) and human MAFbx protein (SEQ ID NO: 41), and human Fbx25 (SEQ ID NO: 42).

Paragraph starting on page 15, line 14:

Figure 32 (SEQ ID NOS: 43 and 44): Sequence alignment demonstrating that MAFbx protein (SEQ ID NO: 43) is the same protein as MA61 (SEQ ID NO: 44), and the different names demonstrate a change in nomenclature.

Paragraph starting on page 15, line 17:

Figure 33 (SEQ ID NOS: 45 and 46): Sequence alignment demonstrating that MuRF1 protein (SEQ ID NO: 45) is the same protein as MA16 (SEQ ID NO: 46), and the different names demonstrate a change in nomenclature.

Paragraph starting on page 15, line 20:

Figure 34 (SEQ ID NOS: 47 and 48): Sequence alignment of rMA16 (SEQ ID NO: 47) with hMURF1 (SEQ ID NO: 48).

Paragraph starting on page 17, line 13:

The invention includes the nucleic acid molecules containing the DNA sequences in Figure 6 (SEQ ID NO: 20), Figures 8A-8C (SEQ ID NO: 22), Figure 10 (SEQ ID NO: 24), Figure 12 (SEQ ID NO: 26), Figure 16 (SEQ ID NO: 32), Figure 18 (SEQ ID NO: 34), and Figure 21 (SEQ ID NO: 38); any DNA sequence that encodes a polypeptide containing the amino acid sequence of Figure 7 (SEQ ID NO: 21), Figure 9 (SEQ ID NO: 23), Figure 11 (SEQ ID NO: 25), Figure 13 (SEQ ID NO: 27), Figure 17 (SEQ ID NO: 33), and Figure 19 (SEQ ID NO: 35); any nucleotide sequence that hybridizes to the complement of the nucleotide sequences that encode the amino acid sequence of Figure 6 (SEQ ID NO: 20), Figures 8A-8C (SEQ ID NO: 22), Figure 10 (SEQ ID NO: 24), Figure 12 (SEQ ID NO: 26), Figure 16 (SEQ ID NO: 32), Figure 18 (SEQ ID NO:

34), and Figure 21 (SEQ ID NO: 38); under stringent or highly stringent conditions, and/or any nucleotide sequence that hybridizes to the complement of the nucleotide sequence that encodes the amino acid sequence of Figure 7 (SEQ ID NO: 21), Figure 9 (SEQ ID NO: 23), Figure 11 (SEQ ID NO: 25), Figure 13 (SEQ ID NO: 27), Figure 17 (SEQ ID NO: 33), Figure 19 (SEQ ID NO: 35), and Figure 22 (SEQ ID NO: 39), under less stringent conditions.

Paragraph starting on page 17, line 22:

In a specific embodiment, the nucleotide sequences of the present invention encompass any nucleotide sequence derived from a mammalian genome which hybridizes under stringent conditions to Figure 10 (SEQ ID NO: 24), Figure 12 (SEQ ID NO: 26), and Figure 18 (SEQ ID NO: 34) and encodes a gene product which contains either an F-box motif and is at least 47 nucleotides in length.

Paragraph starting on page 18, line 13:

In specific embodiments, the invention provides for nucleotide fragments of the nucleic sequences encoding MURF1, MURF3, and MAFBX [Figure 6 (SEQ ID NO: 20), Figures 8A-8C (SEQ ID NO: 22), Figure 10 (SEQ ID NO: 24), Figure 12 (SEQ ID NO: 26), Figure 16 (SEQ ID NO: 32), Figure 18 (SEQ ID NO: 34), and Figure 21 (SEQ ID NO: 38)]. Such fragments consist of at least 8 nucleotides (i.e. hybridization portion) of an MURF1, MURF3, or Mafbx gene sequence; in other embodiments, the nucleic acids consists of at least 25 continuous nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, 150 nucleotides, or 200 nucleotides of an MURF1, MURF3, or MAFBX sequence. In another embodiment the nucleic acids are smaller than 47 nucleotides in length. The invention also relates to nucleic acids hybridizable or complementary to the foregoing sequences. All sequences may be single or double stranded. In addition, the nucleotide sequences of the invention may include

nucleotide sequences that encode polypeptides having at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or higher amino acid sequence identity to the polypeptides encoded by the MURF1, MURF3, or MAFBX sequences of the complement of the nucleotide sequence that encodes the amino acid sequence of Figure 7 (SEQ ID NO: 21), Figure 9 (SEQ ID NO: 23), Figure 11 (SEQ ID NO: 25), Figure 13 (SEQ ID NO: 27), Figure 17 (SEQ ID NO: 33), and Figure 19 (SEQ ID NO: 35).

Paragraph starting on page 18, line 28:

One embodiment of the invention is a recombinant nucleic acid encoding MURF1, MURF3, or MAFBX polypeptide which corresponds to the amino acid sequence as set forth herein in Figure 7 (SEQ ID NO: 21), Figure 9 (SEQ ID NO: 23), Figure 11 (SEQ ID NO: 25), Figure 13 (SEQ ID NO: 27), Figure 17 (SEQ ID NO: 33), and Figure 19 (SEQ ID NO: 35), or a fragment thereof having MURF1, MURF3, or MA-61-specific activity or expression level.

Paragraph starting on page 18, line 33, through page 19, line 2:

Still another embodiment is an isolated nucleic acid comprising a nucleotide sequence as set forth herein in Figure 6 (SEQ ID NO: 20), Figures 8A-8C (SEQ ID NO: 22), Figure 10 (SEQ ID NO: 24), Figure 12 (SEQ ID NO: 26), Figure 16 (SEQ ID NO: 32), Figure 18 (SEQ ID NO: 34), and Figure 21 (SEQ ID NO: 38), or a fragment thereof having at least 18 consecutive bases and which can specifically hybridize with the complement of a nucleic acid having the sequence of native MURF1 or MAFBX.

Paragraph starting on page 21, line 9:

The invention provides for the detection of nucleic acids encoding MURF1, MURF3, and MA-61. This may be done through the use of nucleic acid hybridization probes and replication/amplification primers having a MURF1, MURF3, or MAFBX cDNA-specific sequence and sufficient to effect specific hybridization with Figure 6 (SEQ ID NO: 20), Figures 8A-8C (SEQ ID NO: 22), Figure 10 (SEQ ID NO: 24), Figure 12 (SEQ ID NO: 26), Figure 16 (SEQ ID NO: 32), Figure 18 (SEQ ID NO: 34), and Figure 21 (SEQ ID NO: 38). Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M NaPO₄, pH 7.7, 0.001 M EDTA) buffer at a temperature of 42oC and remaining bound when subject to washing at 42oC with 0.2 x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42oC and remaining bound when subject to washing at 42oC with 0.2 x SSPE buffer at 42oC., or most preferably hybridizing in a buffer comprising 20% SDS, 10% BSA, 1M NaPO₄, .5M EDTA, pH 8 at a temperature of 60oC and remaining bound when subject to washing at 65oC with 2x SSC, .1% SDS. MURF1 or MAFBXcDNA homologs can also be distinguished from one another using alignment algorithms, such as BLASTX (Altschul, et al., (1990) Basic Local Alignment Search Tool, J. Mol. Biol. 215:403-410).

Paragraph starting on page 23, line 19:

One embodiment of the invention is an isolated MURF1, MURF3 or Mafbx polypeptide comprising the amino acid sequence as set forth herein in Figure 7 (SEQ ID NO: 21), Figure 9 (SEQ ID NO: 23), Figure 11 (SEQ ID NO: 25), Figure 13 (SEQ ID NO: 27), Figure 17 (SEQ ID NO: 33), Figure 19 (SEQ ID NO: 35), and Figure 22 (SEQ ID NO: 39), or a fragment thereof having MURF1, MURF3 or MA-61-specific activity or expression levels.

Paragraph starting on page 30, line 13:

The invention further provides for a method for screening for agents useful in the treatment of a disease or disorder associated with muscle atrophy comprising contacting a cell expressing MURF1, MURF3 or Mafbx having the amino acid sequence of Figure 7 (SEQ ID NO: 21), Figure 9 (SEQ ID NO: 23), Figure 11 (SEQ ID NO: 25), Figure 13 (SEQ ID NO: 27), Figure 17 (SEQ ID NO: 33), Figure 19 (SEQ ID NO: 35), and Figure 22 (SEQ ID NO: 39), respectively, or a fragment thereof, and its substrate, with a compound and detecting a change in the activity of either MURF1, MURF3 or Mafbx gene products. Such change in activity may be manifest by a change in the interaction of MURF1, MURF3 or Mafbx gene products with one or more proteins, such as one of their substrates or a component of the ubiquitin pathway, or by a change in the ubiquitination or degradation of the substrate.

Paragraph starting on page 37, line 8:

Northern probes for mouse *myoD* spanned bp 571-938 of coding sequence; mouse *myogenin* spanned bp 423-861 of coding sequence mouse *Myf5* spanned 406-745 of coding sequence. Northern probes for rat *MuRF1* were made by PCR, spanning bp 24 - 612 of coding sequence. For mouse *MuRF2*, the probe was made using the 5' PCR oligo: GAACACAGGAGGAGAACTGGAACATGTC (SEQ ID NO: 1) and the 3' PCR oligo: CCCGAAATGGCAGTATTTCTGCAG (SEQ ID NO: 2), spanning the fifth exon of mouse *MuRF2*. For mouse *MuRF3*, the probe spanned bp 867-1101 of coding sequence. For rat *MAFbx*, the probe was made by PCR, and spanned bp 21 - 563 of coding sequence. For human *MAFbx*, the probe spanned bp 205 - 585. The Northern of mRNA from the *MAFbx* +/+, +/-, and -/- mice was probed with coding sequence spanning bp 660 - 840. To control for the amount of total RNA loaded, the agarose gels were stained with ethidium bromide and photographed, to assess

ribosomal RNA bands. The Southern confirming the loss of the *MAFbx* allele on the 5' end was performed with a mouse *MAFbx* genomic probe, spanning a 1.1 kb *SacII* fragment upstream of the ATG, and downstream of the indicated *EcoRI* site. The Northern of mRNA from the *MuRF1* +/+, +/-, and -/- mice was probed with coding sequence spanning bp 1 - 500 of rat *MuRF1* (accession AY059627). The Southern confirming the loss of the *MuRF1* allele on the 5' end was performed with a mouse *MuRF1* genomic probe, spanning a 0.5 kb *BglII* fragment upstream of the ATG, and downstream of the indicated *EcoRI* site.

Paragraph starting on page 38, line 7:

Rats were subjected to an atrophy-inducing model, as outlined in Example 1 *supra*. Three days after surgery, muscle tissue was harvested from the surgically treated animals. As a control, muscle tissue was also harvested from untreated animals. Messenger RNA was isolated from the atrophied muscle tissue and from the control muscle tissue, and put into a differential display assay. One of the gene transcripts found to be up-regulated during atrophy encompassed a 3', untranslated part of the *MURF1* transcript. This 3' fragment was used to produce a DNA probe, which was used to clone a full-length gene comprising the coding sequence of *MURF1*. Also identified was an smaller, alternate splice form termed the r*MURF1* VRV splice form. This alternate form differ from the full length form at the 3' end, with the full length form being 152 amino acids longer. The alternate splice form has at its carboxy terminus the amino acid sequence "VRV" which is a PDZ-interacting domain (Torres R, Firestein BL, Dong H, Staudinger J, Olson EN, Huganir RL, Bredt DS, Gale NW, Yancopoulos GD (1998) *Neuron*:1453-63). The presence of a PDZ-interacting domain predicts that the protein is able to participate in protein-protein interactions. In contrast, the full length form has other protein interacting domains, for example, an acidic domain containing the amino acid sequence "DEEEEFTEEEEEEDQEE" (SEQ ID NO: 3). the presence of this domain predicts that

this form is also able to interact with other proteins. The nucleotide and deduced amino acid sequences for full length rMURF1 are appended below in Figure 6 (SEQ ID NO: 20), and Figure 7 (SEQ ID NO: 21), respectively. The nucleotide and deduced amino acid sequences for the rMURF1 VRV splice form are appended below in Figure 16 (SEQ ID NO: 32) and Figure 17 (SEQ ID NO: 33) respectively.

Paragraph starting on page 38, line 33, through page 39, line 5:

The rat MURF1 coding sequence was used to isolate human MURF3, by standard molecular biology techniques. This coding sequence has been previously deposited with American Type Culture Collection (ATCC®), as Human MA16 C8 in Stratagene T3/T7 vector, Patent Deposit Designation #PTA-1049, on December 10, 1999. The nucleotide and deduced amino acid sequences for hMURF3 are appended below in Figure 21 (SEQ ID NO: 38) and Figure 22 (SEQ ID NO: 39) respectively. Human MuRF 1 was used to hybridize to rat MURF1, by standard techniques.

Paragraph starting on page 39, line 9:

This experiment was performed in the interest of determining which genes are differentially expressed during conditions of skeletal muscle atrophy. To find such genes, rats were subjected to an atrophy-inducing model, as outlined in Example 1 *supra*. Three days after surgery, muscle tissue was harvested from the surgically treated animals. As a control, muscle tissue was also harvested from untreated animals. Messenger RNA was isolated from the atrophied and from the control muscle tissue, and put into a differential display assay. One of the gene transcripts found to be up-regulated during atrophy encompassed a 3', untranslated part of the MAFBX transcript. This 3' fragment was used to produce a DNA probe, which was used to clone a full-length gene comprising the coding sequence of MA-61, by standard molecular biology techniques. The nucleotide and deduced amino acid

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sequences for rMAFBX are appended below in Figure 10 (SEQ ID NO: 24) and Figure 11 (SEQ ID NO: 25), respectively.

Paragraph starting on page 39, line 24, through page 40, line 2:

The rat MAFBX coding sequence was used to isolate the human homolog of MAFBXD18, by standard molecular biology techniques. Two alternate forms of this gene were identified, termed hMAFBXD18 and hMAFBXK8. The D18 form of the gene encodes a protein which is 11 amino acids longer at the carboxy terminus than the K8 form. The significance of having two forms of this gene is unknown. However, it is often the case that alternate splice forms serve to modulate protein-protein interactions. These coding sequence has been previously deposited with American Type Culture Collection (ATCC®) as Human MAFBXK8 in Stratagene T3/T7 vector, Patent Deposit Designation #PTA-1048 and Human MAFBXD18 in Stratagene T3/T7 vector, Patent Deposit Designation #PTA-1050. The nucleotide and deduced amino acid sequences for hMAFBXK8 are appended below in Figure 12 (SEQ ID NO: 26) and Figure 13 (SEQ ID NO: 27), respectively. The nucleotide and deduced amino acid sequences for hMAFBXD18 are appended below in Figure 18 (SEQ ID NO: 34), and Figure 19 (SEQ ID NO: 35), respectively.

Paragraph starting on page 40, line 4:

The sequences of rat and human MAFbx protein, and human Fbx25 were aligned (C. Cenciarelli *et al.*, *Curr. Biol.* 9, 1177 (1999)). The published partial Fbx25 sequence begins with the indicated Leucine (L) at amino acid 85 of MAFbx. The region surrounding the F-box is indicated, as is a bipartite nuclear localization signal. (Figure 26 [SEQ ID NOS: 40-42]). Accession numbers for rat and human *MAFbx* are AY059628 and AY059629, respectively.

Paragraph starting on page 42, line 25:

These findings indicate that denervation and immobilization are easily distinguishable transcriptionally from unweighting, perhaps because unweighting is unique in that there is relatively normal neural activation and joint movement in the suspended limbs. However, we did identify two genes that were up-regulated in all three models of atrophy; MA16, later identified as MuRF1 (for muscle-specific ring finger protein), and MA61, (subsequently called MAFbx, for Muscle Atrophy F-box protein). (Fig. 2A)

Paragraph starting on page 43, line 17:

Identification of a gene whose expression was up-regulated during atrophy and down-regulated during hypertrophy would greatly strengthen the claim that this gene was a marker for the atrophy phenotype, and provide correlative evidence that the gene of interest may function as a direct mediator of the atrophy process. We therefore examined MuRF1 and MAFbx expression in two models of skeletal muscle hypertrophy: hind-limb reloading following a 14-day unweighting period (D. B. Thomason, R. E. Herrick, D. Surdyka, K. M. Baldwin, *J Appl Physiol* **63**, 130-7. (1987).), and compensatory hypertrophy in which the gastrocnemius and soleus muscles are removed, leaving the plantaris muscle to compensate for the loss of these synergistic muscles (G. R. Adams, F. Haddad, *J Appl Physiol* **81**, 2509-16. (1996); R. R. Roy *et al.*, *J Appl Physiol* **83**, 280-90. (1997). In both of these models, MuRF1 and MAFbx expression decreased, demonstrating that these genes are not only positively correlated with atrophy, but are also negatively correlated with hypertrophy (Fig 28C). Furthermore, Northern analysis on both rat and human "tissue blots" identified MuRF1 and MAFbx as being muscle-specific, in both heart and skeletal muscle (Fig. 28D), consistent with their serving specific roles in these tissues.

Paragraph starting on page 44, line 7:

Recently, it has been shown that genes containing ring domains can function as "monomeric ubiquitin ligases". Under certain conditions, these proteins simultaneously bind a substrate and a ubiquitin ligase, causing ubiquitination and proteasome-mediated degradation of the substrate. In the process, the ring domain protein itself becomes ubiquitinated. A vector encoding the rat MURF1 gene was transfected into COS cells, along with a vector encoding an HA-epitope-tagged form of ubiquitin. Protein lysates were harvested from the COS cells. MURF1 was immune-precipitated from the lysate using an antibody raised against an MURF1 peptide. The immune-precipitated protein was subjected to Western blot analysis, utilizing an antibody to the HA-tag. It was seen that MURF1 is highly ubiquitinated. Further, as a control, a vector encoding a mutant form of MURF1, in which the ring domain portion of the gene was deleted, was co-transfected into COS with tagged ubiquitin. In this case, no ubiquitination was evident. These results are consistent with the hypothesis that MURF1 functions as part of a ubiquitin complex, and that the ring-domain is necessary for ubiquitination, as seen in other ring domain proteins. Figure 14 (SEQ ID NOS: 28-31) is a comparison of hMURF1 with other ring finger proteins.

Paragraph starting on page 47, line 23, through page 48, line 8:

Two genes closely related to MuRF1 have been cloned, and named MuRF2 and MuRF3, (T. Centner *et al.*, *J Mol Biol* **306**, 717-726 (2001), J. A. Spencer, S. Eliazar, R. L. Ilaria, J. A. Richardson, E. N. Olsen, *J. Cell Biol.* **150**, 771-784 (2000)). Northern analysis demonstrated that MuRF2 and MuRF3 expression were not consistently up-regulated during skeletal muscle atrophy (Fig 4C), despite being muscle specific and highly homologous to MuRF1 (T. Centner *et al.*, *J Mol Biol* **306**, 717-726 (2001)).

Muscle was obtained from rats undergoing a time course (0, 1, 3, and 7 days) of three atrophy models: immobilization, denervation, and hindlimb-suspension. For each lane, total RNA was pooled from three rat medial gastrocnemius muscles (MG). Northern hybridizations were performed with probes for the indicated genes. Northern probes for mouse *myoD* spanned bp 571-938 of coding sequence; mouse *myogenin* spanned bp 423-861 of coding sequence mouse *Myf5* spanned 406-745 of coding sequence. Northern probes for rat *MuRF1* were made by PCR, spanning bp 24 - 612 of coding sequence. For mouse *MuRF2*, the probe was made using the 5' PCR oligo: GAACACAGGAGGAGAACTGGAACATGTC (SEQ ID NO: 4) and the 3' PCR oligo: CCCGAAATGGCAGTATTTCTGCAG (SEQ ID NO: 5), spanning the fifth exon of mouse *MuRF2*. For mouse *MuRF3*, the probe spanned bp 867-1101 of coding sequence. To control for the amount of total RNA loaded, the agarose gels were stained with ethidium bromide and photographed, to assess ribosomal RNA bands. It is unknown whether *MuRF2* or *MuRF3* function as ubiquitin ligases.

Paragraph starting on page 49, line 32, through page 50, line 7:

To further elucidate the function of MAFbx we genetically engineered a MAFbx null allele in mice, in which genomic DNA spanning the ATG through the exon encoding the F-box region was replaced by a LacZ/neomycin cassette, (Figure 27A) allowing us to simultaneously disrupt MAFbx function and perform b-galactosidase (b-gal) staining to determine MAFbx expression patterns. Analysis of the MAFbx locus demonstrated the expected perturbation in MAFbx +/- and -/- animals. Further, MAFbx -/- animals were null for MAFbx mRNA. MAFbx -/- mice were viable, fertile and appeared normal. Mice deficient in MAFbx had normal growth curves relative to wild type litter mates, and skeletal muscles and heart had normal weights and morphology (data not shown).

Paragraph starting on page 50, line 8:

Given the absence of an obvious phenotype, we decided to challenge the mice in an atrophy model to determine the role, if any, of MAFbx in producing skeletal muscle loss. Muscle atrophy was induced by cutting the sciatic nerve, resulting in denervation and disuse of the tibialis anterior and gastrocnemius muscles. Denervation resulted in up-regulation of the MAFbx gene locus in all muscle fibers, as demonstrated by β -gal staining in the tibialis anterior of MAFbx +/- mice (Fig. 31A). Significant muscle atrophy occurred in the tibialis anterior and gastrocnemius muscles of wild type, MAFbx +/+, mice at 7 and 14 days following denervation (Fig 31B). Mice deficient in MAFbx (MAFbx -/-) had significantly less atrophy than MAFbx +/+ mice at both 7 and 14 days (Fig 31B). In fact, MAFbx -/- mice exhibited no additional muscle loss between 7 and 14 days, whereas MAFbx +/+ continued to lose mass. The preservation of muscle mass at 14 days was also reflected in a preservation of mean fiber size and fiber size variability; MAFbx -/- mice had significantly larger fibers than the MAFbx +/+ mice, and maintained the same fiber size variability as seen in the undenervated limb (Fig 31C). These data provide strong evidence that MAFbx is a required regulator of muscle atrophy, and that it may play an important role in the degradation of muscle proteins.

Please replace the paragraph starting on page 50, line 33, through page 51, line 6:

To further elucidate the function of MuRF1 we genetically engineered a MuRF1 null allele in mice, in which genomic DNA spanning the ATG through the exon encoding the F-box region was replaced by a LacZ/neomycin cassette, (Fig 27B) allowing us to simultaneously disrupt MuRF1 function and perform b-galactosidase (b-gal) staining to determine MuRF1 expression patterns. Analysis of the MuRF1 locus demonstrated the expected perturbation in MuRF1 +/- and -/- animals. Further, MuRF1 -/- animals were null for MuRF1 mRNA. MuRF1 -/- mice were viable,

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fertile and appeared normal. Mice deficient in MuRF1 had normal growth curves relative to wild type litter mates, and skeletal muscles and heart had normal weights and morphology (data not shown).

IN THE CLAIMS:

Claim 1:

1. An isolated nucleic acid molecule comprising a nucleotide sequence which encodes a protein comprising the amino acid sequence as set forth in SEQ ID NOS: 25, 27, or 35.

Claim 2:

2. An isolated nucleic acid molecule which encodes MAFBX, or a fragment thereof, having a sequence selected from the group consisting of

a) the nucleotide sequence comprising the coding region of MAFBX as set forth in SEQ ID NOS: 24, 26, or 35.

(b) a nucleotide sequence who complement hybridizes under stringent conditions to the nucleotide sequences of (a) and which encodes a molecule having the biological activity of MAFBX; or

(c) a nucleotide sequence which, but for the degeneracy of the genetic code would hybridize to a complement of the nucleotide sequence of (a) or the complement of (b), and which encodes a molecule having the biological activity of MAFBX.

Claim 3:

3. An isolated nucleic acid molecule which is derived from a mammalian genome that:

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a) hybridizes under stringent conditions to the nucleic acid molecule of SEQ
ID NOS: 24, 26, or 34; and

b) encodes a gene product which contains a ring domain